

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
17 February 2005 (17.02.2005)

PCT

(10) International Publication Number  
**WO 2005/014636 A1**

(51) International Patent Classification<sup>7</sup>: **C07K 14/47**,  
14/435

(21) International Application Number:  
PCT/EP2004/008739

(22) International Filing Date: 4 August 2004 (04.08.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
03016691.2 4 August 2003 (04.08.2003) EP

(71) Applicant (for all designated States except US): **MAX-  
PLANCK-GESELLSCHAFT ZUR FÖRDERUNG  
DER WISSENSCHAFTEN E.V.** [DE/DE]; Hofgarten-  
strasse 8, 80539 München (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **GRIESBECK,**  
**Oliver** [DE/DE]; Kellerstrasse 31, 81667 München (DE).  
**HEIM, Nicola** [DE/DE]; Bocksteinerstrasse 41, 81241  
München (DE).

(74) Agent: **WICHMANN, Hendrik**; Isenbruck Bösl  
Hörschler Wichmann Huhn Prinzregentenstr. 68, 81675  
München (DE).

(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,  
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,  
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,  
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,  
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,  
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,  
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,  
ZW.

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,  
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,  
GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report
- before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: **GENETICALLY ENCODED BIOINDICATORS OF CALCIUM-IONS**

(57) Abstract: The present invention relates to novel types of cellular calcium probes that are based on Troponin C and two chromophors suitable for FRET (fluorescence resonance energy transfer). The Troponin C-based calcium sensors of the invention function in diverse subcellular environments, for example even when tethered to a cellular membrane. The invention further provides nucleic acid constructs encoding the calcium probes of the invention, expression constructs, host cells and transgenic animals. Furthermore, methods for the detection of changes of local calcium concentrations and for detecting the binding of a small molecule to fragments of Troponin C are provided.

WO 2005/014636 A1